Glutamate dehydrogenase is a complex enzyme with a central role in metabolism, and it has been purified from many species of micro-organisms and animals and studied in detail. Different glutamate dehydrogenases can be classified according to (1) substrate specificity, being NAD-dependent, NADP-dependent or of dual specificity, and (2) the range of effector molecules that modify the activity. The latter include, in any one species, some of the following: dicarboxylic acids, citrate, EDTA, glutamine, various nucleotides, bivalent cations, acyl-CoA, sugar phosphates, hormones, and also some substrates, which show allosteric effects. The role in vivo of most of these regulatory properties is not clear. Some regularity emerges from a classification of glutamate dehydrogenases based on these properties, but as yet there is no clear suggestion of the number of distinct types in micro-organisms.

In most species for which reliable molecular-weight measurements are available the enzyme is a hexamer of molecular weight about 300000, the subunit molecular weight being about 50000. The property of polymerization of the hexamer into indefinite aggregates of higher molecular weight has been found in only a few species, e.g. in the bovine, chicken and frog liver enzymes (Frieden, 1971). In Neurospora crassa, which like many other higher fungi and some bacteria has two distinct glutamate dehydrogenases, one NAD-dependent and the other NADP-dependent, the NADP-dependent enzyme is a hexamer, as outlined above (Wootton et al., 1972), but the NAD-dependent enzyme appears to be different in molecular weight and other properties (E. L. Smith, personal communication).

Comparisons of amino acid sequences show considerable evolutionary conservatism in glutamate dehydrogenases. The bovine liver glutamate dehydrogenase subunit, the sequence of which has been very nearly completely determined (Moon et al., 1972), is a chain of 500 residues. Large parts of this sequence are completely identical in the chicken liver enzyme, for which only 30 amino acid differences, including an additional three residues at the N-terminus, are reported (Moon et al., 1972). From the Neurospora NADP-dependent glutamate dehydrogenase we have deduced from tryptic, chymotryptic and peptic peptides fragments of the sequence that together make up nearly 500 residues. Many of these fragments show significant homology with parts of the bovine enzyme, which suggests a remote common evolutionary origin of the fungal and animal enzymes followed by divergence of substrate specificity and regulatory properties (Wootton et al., 1973).

The Neurospora NADP-dependent glutamate dehydrogenase is of interest because of the existence of many mutants producing functionally distinct enzymes (Fincham, 1962).
These may be used to investigate the role of parts of the molecule containing amino acid substitutions. This approach is complementary to the study of chemical modifications of reactive residues. Of the mutants available some produce no detectable glutamate dehydrogenase activity or protein. These include two chain-terminating mutants (which produce modified glutamate dehydrogenase in suppressor strains), and one apparent frame shift (which reverts at high frequency with the acridine compound ICR 170 to give revertants with modified glutamate dehydrogenases). Mutants with protein that closely resembles wild-type glutamate dehydrogenase in structure include the following classes.

1. The protein has no detectable glutamate dehydrogenase activity but probably has a conformation closely resembling the active state of wild-type enzyme, as shown by its ability to form hybrid molecules with subunits of other mutants of defective conformation and to confer on the latter an active state. This class of mutants may contain substitutions with fairly localized effects on the active site. Two such mutants are closely linked on the genetic map (Fincham, 1967).

2. Proteins that potentially have glutamate dehydrogenase activity, but that under physiological conditions are abnormally stable in inactive states and are activated only very slowly (in the order of minutes to hours) by extremes of conditions that normally activate the wild-type enzyme. The effect of the amino acid substitutions in this class of mutants must be either to decrease the stability of the active conformation or to slow down conformation changes necessary for activation. These mutations occur in two distinct regions of the genetic map.

3. Enzymes derived by reversion of class 2 mutants above, in which a further amino acid substitution (possibly of the same or a different residue) has restored glutamate dehydrogenase activity to a state sufficient to function in vivo, but in which the enzyme is distinct from the wild-type enzyme in one or more of its properties. From a single class 2 mutant a series of such revertants may be obtained with properties ranging from close to the original mutant to close to the wild-type enzyme.

The amino acid substitutions have been identified in two class 2 mutants am² and am¹⁹, which have similar properties but differ in their requirements for activation. In mutant am¹⁹ a lysine residue is replaced by methionine, and in mutant am² (very closely linked to mutant am¹⁹ genetically) the histidine residue immediately next to this lysine residue towards the C-terminus is replaced by glutamine. These residues are part of a basic sequence -His-Lys-His-, which aligns, as part of a homology extending over 40 residues, with a basic -Lys-Lys- sequence (residues 154-155) in bovine glutamate dehydrogenase. Thus the positive charges on this part of the molecule may be necessary for the fully active conformation. In strain R24, a class 3 revertant from mutant am¹⁹, whose glutamate dehydrogenase resembles that of mutant but is more readily activatable, the original substitution of methionine for lysine is still present and there is also a substitution of arginine for glutamine in a different part of the chain. This second replacement may restore a positive charge close to the replaced lysine residue in the three-dimensional structure of the enzyme.

Another homology between Neurospora NADP-dependent glutamate dehydrogenase and the bovine enzyme occurs in a length of 30 residues that includes lysine-126 of the bovine enzyme, this residue being highly reactive to pyridoxal 5′-phosphate and cyanate (Piszkiiewicz et al., 1970). This region is also homologous with part of the sequence of porcine glyceraldehyde 3-phosphate dehydrogenase including lysine-212, which also reacts with pyridoxal 5′-phosphate. The functional significance of the reactivity of these lysine residues is not clear. The other known reactive residues of bovine glutamate dehydrogenase are in a part of the sequence for which there is no obvious homology with the Neurospora enzyme. These are tyrosine-406, which is readily nitrated with tetranitromethane with concomitant loss of sensitivity to inhibition by GTP, and lysine-419 and lysine-422, which are highly reactive to trinitrobenzenesulphonate in a mutually exclusive manner (Goldin & Frieden, 1971). Since the Neurospora enzyme does not bind GTP, it is possible that the lack of homology in this part of the chain reflects functional divergence.

Since a deeper understanding of the functioning of glutamate dehydrogenase will depend on the determination of the structure by X-ray crystallography, attempts have
been made to grow a variety of suitable crystal forms. Preliminary crystallographic data are available for a trigonal form of the Neurospora NADP-dependent enzyme, which has a complex unit cell. It is likely that a complete structure determination will be a very major undertaking.


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Crystallographic Studies of Triose Phosphate Isomerase

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**Vitamin B12: Enzyme Catalysed Isomerizations**

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**COMMUNICATIONS**

**Multiple Forms of Cyclohexanone Oxygenase from Nocardia globerula CL1**

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Cyclohexanone oxygenase is one of the induced enzymes involved in the metabolism of cyclohexanol by *Nocardia globerula* CL1 (Norris & Trudgill, 1971). It catalyses the conversion of cyclohexanone into 1-oxa-2-oxocycloheptane with a unimolar consumption of O₂ and NADPH, indicating that it, like the cyclopentanone oxygenase of *Pseudomonas* N.C.I.B. 9872 (Griffin & Trudgill, 1972), is a mixed-function oxygenase catalysing a ring-oxygen insertion rather than the formation of a hydroxyl group.

Norris & Trudgill (1972) have reported the purification of this enzyme to what was believed to be homogeneity. It yielded a single protein band on polyacrylamide-gel electrophoresis in 2.25nm-average-pore-radius gels (Gordon, 1969) and a symmetrical sedimentation peak in the analytical ultracentrifuge with $s_{20,w}^0$ 3.9S; a $D_{20,w}$ value of 6.87 was obtained and the molecular weight calculated from these data and from an approach-to-equilibrium experiment was 53000. This enzyme was bright yellow, displayed absorption maxima at 278, 367 and 435nm and was calculated to bind 1 mol of FAD/53000g and to possess one catalytically functional thiol group/molecule.